It is known that several bacteria (primarily pseudomonads) produce medium-chain-length (mcl) PHAs from fatty acids (5-7). However, only recently have intact triglycerides been considered as feedstocks for PHA production. Three bacterial species have been shown to produce PHA from triglycerides. These include *Aeromonas caviae,* which produce a copolymer of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) from olive oil (7), *Pseudomonas aeruginosa,* which produce a complex PHA copolymer when grown on euphorbia or castor oil (9), and *Pseudomonas resinovorans,* which produce a PHA from tallow (8). In our laboratory we have investigated the use of *P. resinovorans* to synthesize unique PHAs from a variety of triglyceride substrates.

Results and Discussion
Six triglyceride substrates (lard, butter oil, olive oil, high oleic acid sunflower oil, coconut oil, and soybean oil) were screened as potential substrates for PHA production. A two-stage fermentation was used to increase the number of viable cells prior to transfer into the polymer production medium in hopes that increased PHA yields could be achieved. Each triglyceride, whether animal fat or vegetable oil, supported cell growth **(Table 1).** This indicated that the organism showed no significant preference towards fats (solids) or oils (liquids) as substrates for growth and polymer production. After 48 h in the polymer production medium the cells were viewed under a phase-contrast microscope for the presence of phase-bright inclusions, evidence of polymer production. *P. resinovorans* produced an mcl-PHA from each triglyceride. This was evident by the presence of one or more PHA granules per bacterium that, when visually inspected, appeared to constitute approximately 50% of the cell mass. The cells were harvested by centrifugation and the cellular biomass, and PHA content and yield were determined **(Table 1)**. The average PHA content for all tested triglycerides was 45%, and the average PHA yield was about 1.5 g/L. Thus, our two-stage fermentation system resulted in a 200% increase in PHA production compared to previously reported results (6). Repeat-unit composition analysis by gas chromatography of the tallow-derived PHA showed that the predominant monomers of the polymers were 3-hydroxyoctanoate and 3-hydroxydecanoate. The number-average molecular mass of the PHA-tallow was 87-93 kDa as determined by gel permeation chromatography. Similarly, *Ps. saccharophila,* which produces a high level of extracellular lipase activity [10], was shown to grow and produce PHA in medium containing tallow or coconut oil as a sole carbon source [11]. Transmission electron microscopic study of the tallow-grown *Ps. saccharophila* showed the presence of intracellular inclusion bodies characteristic of PHA-producing bacteria **(Figure 1)**. Ashby and Foglia [8] further characterized the utilization of a series of agricultural lipids by *Ps. resinovorans* for cell growth and mcl-PHA production. **Table 1** shows that the organism yielded cell biomass in the range of 2.4-4.1 g cell-dry-weight (CDW) per liter (L) culture when grown with animal fat or vegetable oil as a sole carbon source. The PHA contents of these cells ranged from 43-59% of CDW. More significantly, repeat-unit (β-hydroxy fatty acids) composition analysis of the polymers showed that the chemical structures of the pendant alky groups are highly dependent on the feedstock. When coconut oil, which contains predominantly saturated fatty acids, was used as a substrate, the resulting PHA polymers have repeat-units with virtually no detectable double-bond functional groups **(Table 2)**. On the other hand, soybean oil-

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Polyester Polymer Production by Microorganisms Grown on Fat and Oil Substrates

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Abstract

The fermentation of fats and oils to produce biodegradable polymers is a potential growth area for utilization of these commodities. Consumers are becoming more environmentally aware of the consequences of disposal of waste plastic materials, as evidenced by recycling efforts in many communities. Many plastics (*e.g.*, garbage bags, and disposable diapers) remain in landfill sites for many years because of their limited biodegradation by soil organisms. Accordingly, the production and use of environmentally benign biopolymers is a growing opportunity. Many microorganisms can produce environmentally compatible polymers, but few produce sufficient amounts of the polymers to make their use economically feasible. One feasible class of biopolymers is poly(hydroxyalkanoate) polyester (PHA). Bacteria produce PHA's when grown on a variety of carbon substrates including animal fats, vegetable oils, and waste greases. Triglyceride-based PHA's can be processed to naturally biodegradable materials that exhibit properties similar to many petroleum-based polymers. Their chemical makeup, which is dictated both by the enzymes present in the bacteria as well as the carbon substrates on which they are grown, governs the properties of individual PHA's. Here, we describe the production of a series of PHA's synthesized by wild type and genetically altered pseudomonad bacteria grown on a variety of fat and oil substrates. The physical properties of these medium-chain-length PHA polymers vary from elastomeric to amorphous. This directs their potential applications. The presence of olefinic groups in the PHA polymer side-chains, inherited from the olefinic content of the triglyceride feedstock, provides loci for chemical modifications to enhance the polymer properties and expand their use to other applications.

Introduction

Poly(hydroxyalkanoates) (PHAs) are naturally occurring, optically active polyesters that accumulate in numerous bacteria as carbon and energy storage materials (1-3). In most cases the polymers contain β-linked repeat units and possess the general structure:

$$[\text{-O-(CHR)-CH}_2\text{-CO-}]_x$$

The R group varies, from C_1-C_{13}, based on the bacterium and the carbon substrate from which the polymer was formed (4). Recently, there has been significant interest in the use of PHAs for biodegradable thermoplastics. Because they are viewed as "environmentally friendly," they are being studied as potential replacements for synthetic plastics in several applications. One major drawback to the use of these polymers is the cost involved in production. Generally, the cost to produce a given PHA polymer on an industrial scale is greater than for a comparable synthetic polymer. To make PHA production more commercially viable, two avenues can be pursued: produce PHAs whose properties allow for their use in unique applications; and lower the production costs either by increasing polymer yields or by using less expensive substrates. The latter possibility (and to some extent the former) can be achieved by using agricultural triglycerides as carbon substrates.

derived PHA polymers contain a high degree of unsaturation in the pendant alkyl groups **(Table 2)**. A wide range of repeat-units is observed because the metabolic process that produces the polymers also shortens the fatty acid chains.

Conclusion

In summary, we have demonstrated that *Ps. resinovorans* and *Ps. saccharophila* are able to grow and synthesize PHA by using the inexpensive agricultural fats and oils as substrate. Furthermore, manipulating the type of feedstock used in the fermentation can modify the chemical composition and thus the physical properties of these biodegradable polymers.

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TABLE 1. Cell Dry Weights and Poly(hydroxyalkanoate) Polymer Content and Yields of *P. resinovorans* Grown on Triglyceride Substrates

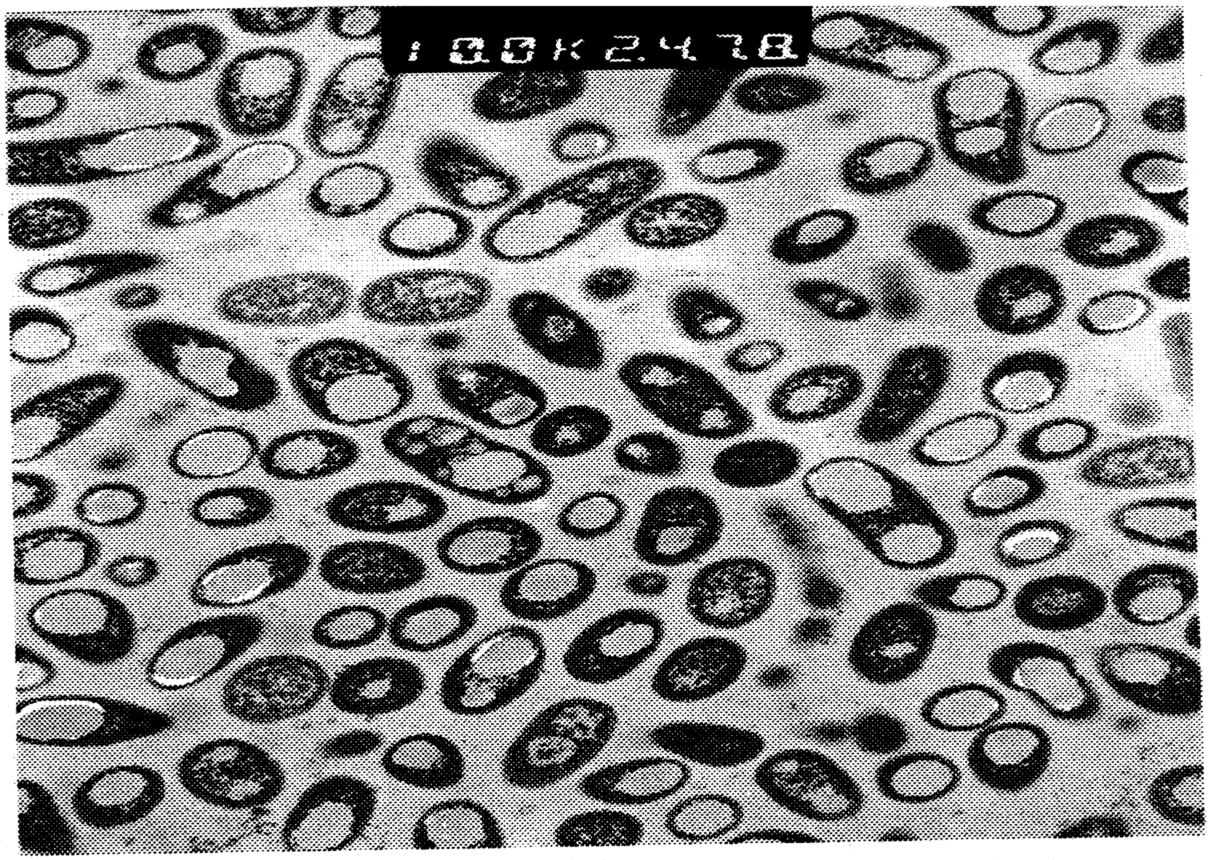
Substrate	Cell Yield[a] (g/L)	PHA content[b] (% dry weight)	PHA yield[c] (g/L)
Control			
Oleic acid	3.8	43	1.9
Animal fats			
Tallow	3.0	39.8	2.1
Lard	3.6	47.4	1.7
Butter oil	3.6	47.0	1.7
Vegetable oils			
Olive	3.4	43.1	1.5
Sunflower (high oleic)	3.1	41.2	1.3
Coconut	3.8	51.0	1.9
Soybean	2.9	44.5	1.3
Averages[d]	3.3	44.9	1.5

[a]Cell dry weight average yields (n = 3). [b]PHA per cell dry weight averages (n = 3). [c]Calculated by multiplying the cell yield (g/L) by the PHA content (% dry weight) of the cells. [d]Averages do not include oleic acid values.

TABLE 2. β-Hydroxy fatty acid repeat unit composition of poly(hydroxyalkanoate) polymers produced by *P. resinovorans* grown on triglyceride substrates.

	β-hydroxymethyl ester (%)[a]									
PHA-Substrate	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{14:2}$	$C_{14:3}$
Tallow	Tr	3	15	46	17	4	6	9		
Lard	Tr	7	26	34	14	4	4	8	3	
Butter	Tr	9	31	35	15	Tr	4	5		
Olive	1	8	29	33	14	1	3	10	1	
Sunflower	2	5	22	35	14	3	3	13	3	
Soy	Tr	4	18	32	8	14	4	9	10	Tr
Coconut	Tr	7	33	40	16	1	3	Tr		

[a]Data are gas chromatographic (GC) area percents of methyl esters of the β-hydroxy acids produced by the acid hydrolysis of the PHA polymers found in Table 1.



Ps. saccharophila **NRRL B-628 (Exp. DKS-I-89)**

Figure 1. Thin-section electron microscopic image of *Ps. Saccharophila* grown in a chemically defined medium containing tallow as the sole carbon source.